FIBRINOLYTIC ACTIVITY OF HUMAN BRAIN AND CEREBROSPINAL FLUID

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SUMMARY.—Fibrinolytic activity (FA) of brain tissue, meninges and choroid plexus from 41 human cadavers without intracranial disorders was studied by Astrup's biochemical method and Todd's histochemical method. FA of cerebrospinal fluid (CSF) before and after pneumoencephalography (PEG) was also studied by Astrup's method.

FA of human brain was higher in the adults than in the newborn and infants, and increased with ageing in infants. No significant difference was found among age groups in the adults.

There was no detectable difference of FA in various regions of the brain. Higher FA was recognized in meninges and choroid plexus. Liquefaction of the extravasated blood in the subarachnoid space was considered to be produced by the high fibrinolytic activity of the meninges.

The lysed zones on fibrin plate by Todd's method were found at the vessels of the brain tissue and meninges, especially at small blood vessels. FA was found to be localized at the vascular endothelial cells. The lytic areas in the adult brain were relatively larger than those in the newborn brain at the same incubation time.

CSF produced small lysed zones on human fibrin plate. CSF and plasma after PEG showed larger lysed zones than those before PEG, and plasminogen activator and/or proactivator in CSF and plasma seemed to be increased after PEG. Plasmin activity was not found in CSF before and after PEG.

RECENT investigations of the fibrinolytic activity (FA) of tissues have shown that a large amount of plasminogen activator is present in highly vascular connective tissues, especially in the endothelial cells of the small blood vessels (Kwaan, 1966). FA in tissue is generally considered to be related to the mechanism regulating fibrin deposition in tissue repair (Astrup, 1966) and permeability of blood vessels (Watabe, 1960) and furthermore to the evolution and development of arteriosclerosis (Onoyama, 1967), myocardial infarction (Kwaan, 1966), cirrhosis of the liver (Astrup, Rasmussen, Amery and Poulsen, 1960) and other diseases such as hyaline membrane disease (Lieberman, 1959).

FA of various human tissues was observed by Astrup and Albrechtsen (1957), Ende and Auditore (1961) and Sandberg, Regai, Bangayan, Bellet, Feinberg and Hunter (1963), but scanty study was performed on the FA of the human brain tissue except for the reports of Fantl and Fitzpatrick (1950), Albrechtsen (1957), Tanaka (1960) and Okada, Tsuchiya, Tada, Mishima and Suzuki (1966).

The fibrinolytic enzyme system was presumed to have some relationship to the development of the cerebral vascular diseases, to the blood–brain barrier, and to the haemorrhagic diathesis in newborn infants.

The present study was carried out to elucidate the development and distribution

of FA in the human brain and to discuss its significance in cerebrovascular disease in adult and newborn. FA of cerebrospinal fluid (CSF) before and after pneumoencephalography (PEG) was also reported.

MATERIALS AND METHODS

Fresh samples of human brain, meninges, choroid plexus and cerebral arteries were obtained from 41 cadavers without intracranial disorders autopsied within 12 hr after death. The 41 cases consisted of 6 cases who survived less than 2 days, 4 cases who survived 4–6 days, 6 cases who survived 2–6 months, 7 cases who survived 1–20 yr and 18 cases who survived more than 20 yr. Specimens of human brain were taken from cortex, white matter, basal ganglia (putamen), thalamus and pia mater. These tissue specimens were investigated immediately after their removal or were kept frozen at $-20°$ for later use.

CSF was obtained from 15 patients during lumbar PEG. These patients were diagnosed as cerebral palsy, infantile spasm, cerebral lipidosis and other cerebral degenerative diseases. Samples of CSF and plasma were taken simultaneously before and after PEG in 5 of 15 cases.

1. *The biochemical estimation of FA of human brain* was carried out according to the method reported by Astrup *et al.* (1952, 1957), 0·1 g. of fresh tissue fragment was homogenized and extracted with 3 ml. of 2 M potassium thiocyanate (KSCN). After extraction, the sediment was removed by centrifugation at 2000 rpm for 10 min. The active substance in the super-natant was precipitated by adding 2 ml. of 1 N-HCl. The precipitate was redissolved in 1 ml. of 2 M KSCN. Drops of this tissue extracts (0·03 ml.) were pipetted in duplicate on one side of the unheated human fibrin plate, while similar drops of the control pig heart solution (Astrup and Albrechtsen, 1957) were placed on the opposite side of the plate. The plates were then incubated for 18 hr at 37°, and the sizes of the lysed zones on the fibrin plates were estimated by the product in square millimetres of two perpendicular diameters. The FA of each tissue was expressed as pig heart units, namely the ratio of the area of lysis produced by the test solution to that of the control pig heart solution on the same plate. Fibrin plates were prepared by the method of Astrup and Mullerty (1952) using bovine fibrinogen (Armour Pharmaceutical Co., U.S.A.) or human fibrinogen (Green Cross Co., Japan).

2. *The localization of the FA in the brain tissues* was examined with Todd's method (Todd, 1959) by a " fibrin plate technique ". Frozen sections (8 μ) of fresh human brain tissues were placed directly on the human fibrin plate prepared on the slide glasses. The preparations were then incubated at 37° in a moist chamber for 10, 20, 30, 40, 60 and 80 min. respectively. They were then fixed by exposure to formalin vapour for 12–24 hr, after which they were stained with Harris haemalum and were mounted in glycerine-jelly.

3. *FA of CSF.*—A sample (0·03 ml.) of CSF was placed on the unheated and heated (80° for 30 min.) human or bovine fibrin plates, and then the lysed zones were estimated according to the method of Astrup. Plasminogen activator and proactivator were activated by streptokinase (SK).

RESULTS

Biochemical estimation of FA of human brain

The FA (tissue activator of plasminogen) of the brain tissues in different ages and in various parts of the brain (cortex, white matter, putamen, thalamus,

TABLE I.—*Plasminogen Activator Activity of the Human Brain*

	0–6 days	2–12 months	1–20 yr	21–70 yr
Cerebral cortex	96±16·9 (10)	143±31·2 (6)	240±47·8 (7)	300±70·8 (19)
Cerebral white matter	115±25·9 (10)	163±23·0 (6)	210±50·1 (6)	345±81·4 (15)
Basal ganglia (Putamen)	145±32·6 (8)	171±35·2 (6)	265±61·8 (5)	367±74·8 (19)
Thalamus	157±38·9 (5)	185±36·3 (6)	274±50·8 (4)	333±67·5 (5)
Pia mater	360±65·4 (7)	394± 2·6 (3)	616±69·0 (3)	709±162·9 (17)
Choroid plexus	278±34·0 (4)	381±21·0 (3)	—	—
Middle cerebral arteries	222±19·7 (4)	299 (1)	505±170 (3)	505±137·4 (11)

Mean±SD.
( ) Refers to the number of cases.

pia mater and choroid plexus) was summarized in Table I and Fig. 1. The FA was lower in newborn brain than in adult one ($P < 0.05$), and was increased with age. The values of the FA in adult brain extracts showed a wide range of variation, and there was no significant difference among each age group, but the activity in the 60th decade seemed to be slightly lower than that of adult cases less than 60 yr old. Cerebral cortex, white matter, putamen and pia mater revealed almost the same tendency as shown in Fig. 1.
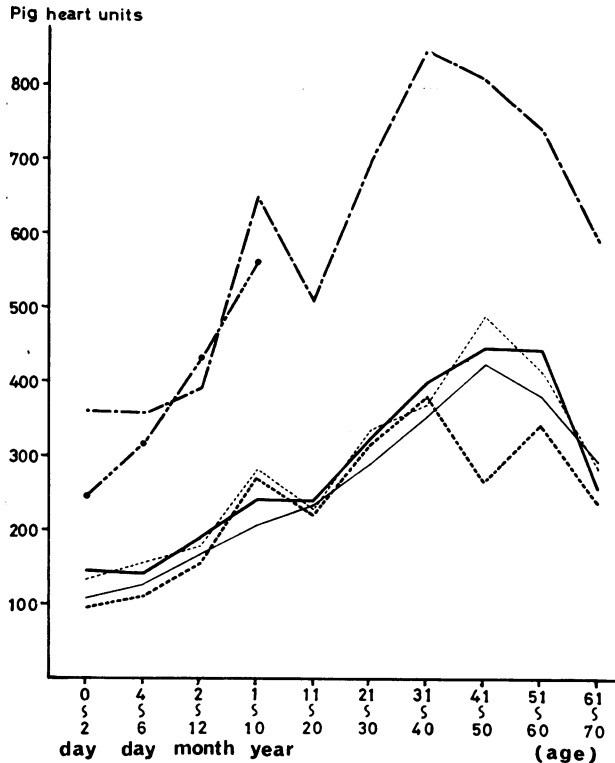

FIG. 1.—Distribution of plasminogen activator in the human brain.

· · · · · · cortex   ———— white matter
– – – – – basal ganglia   ▬▬▬▬ thalamus
—·—·—· pia mater   ···—···— choroid plexus

No significant difference was found among different regions of the human brain, but much higher activity was illustrated in pia mater and choroid plexus ($P < 0.05$). The FA of cerebral arteries was intermediate between brain substance and meninges, though the number of cases was small. The FA of dura mater in 3 newborn cases was 351 ± 122.2 pig heart units.

Histochemical demonstration of the tissue activator by Todd's method

The FA was demonstrated as the area produced by the lysis of fibrin membrane, and was localized at the blood vessels, occasionally at the endothelial cells of small vessels in both of the brain and meninges.

The lysed zones in the adult brain appeared within shorter incubation time than those in the newborn one. The lysed areas were larger in the adult than in the newborn at the same incubation time, though the considerable deviation of FA was seen in the adult. There was no remarkable difference of FA in the various regions of the brain (cortex, white matter, putamen and thalamus). Meninges and choroid plexus showed larger lysed zones than the brain substance at the same incubation time.

FA of CSF

The FA of CSF and plasma obtained before and after PEG is summarized in Tables II and III. CSF before PEG produced small lytic zones on non-heated human fibrin plates, but produced no lytic zones on heated fibrin plates. Hence there seemed to be no plasmin in CSF. CSF after PEG produced larger zones of fibrinolysis on the non-heated human and bovine fibrin plate but no lysed zones on the heated human and bovine fibrin plates. Therefore the FA of CSF seemed to be elevated after PEG, but plasmin was not found after PEG. In CSF added with streptokinase, the lysed zones on the bovine fibrin plate were larger after PEG than before PEG, and therefore plasminogen activator and/or pro-activator seemed to be released in CSF after PEG. FA of plasma before and after PEG in 5 cases showed also the same results as seen in CSF (Table III).

DISCUSSION

The brain is an organ of high thromboplastic activity (Astrup, 1965). The term cerebral vascular diseases includes intracranial haemorrhage such as subarachnoid or subependymal haemorrhage in the newborn infants, and cerebral haemorrhage, cerebral infarction, subarachnoid haemorrhage or subdural hematoma in adults. The FA of the brain was supposed to play some role as a local factor in their pathogenesis, but scanty studies were found in the literature in relation to the FA of the brain tissue. It might be worthwhile to observe those activities of normal and diseased brain tissues biochemically and histochemically. The results obtained in our laboratory revealed that the cerebral artery of the adults had moderate FA in its intimal layer and such an activity was inferred to have some relation to the development of thrombosis and atherosclerosis (Onoyama, 1967). On the other hand, the origin of the FA of CSF was obscure and Porter, Acinapura, Kapp and Silver (1966) supposed its origin in the meninges.

In the present study the age difference and the distribution of the FA in various regions of the human brain were studied by means of Astrup's biochemical method and Todd's histochemical method. The FA of CSF was also studied before and after PEG.

EXPLANATION OF PLATES

FIG. 2.—Lytic zones appeared at the small blood vessels in cerebral cortex of the newborn infant after 80 min. incubation. Harris' alum hematoxylin. × 54.

FIG. 3.—Lytic zones appeared in cerebral cortex of the adult after 60 min. incubation. Harris' alum hematoxylin. × 54.

FIG. 4.—Lytic zones appeared in pia mater and cerebral cortex in relation to the small blood vessels after 40 min. incubation. Harris' alum hematoxylin. × 54.

FIG. 5.—Lytic zones appeared at the small arteries in the white matter of the newborn infant after 40 min. incubation. Harris' alum hematoxylin. × 110.
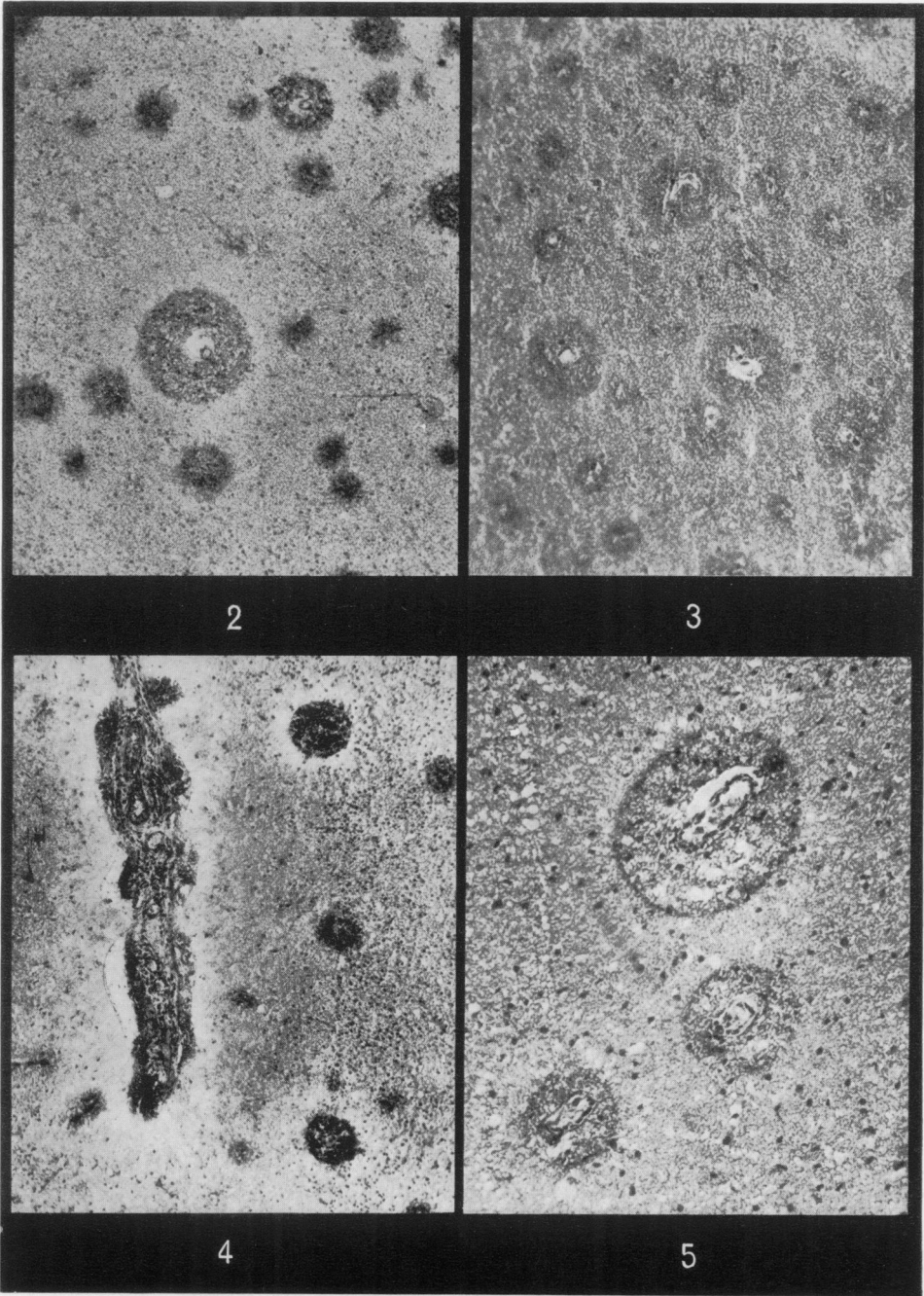
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TABLE II.—*Fibrinolytic Activity of CSF Before and After Pneumoencephalography*

No. of cases	Non-heated plate		Heated plate	
	Before	After	Before	After
1	20·25	50·05	0	0
2	36·00	44·88	0	0
3	25·00	35·75	9·00*	15·20*
4	28·00	36·00	0	0
5	27·04	36·00	0	0
6	49·00	60·00	0	0
7	36·00	56·25	0	0
8	49·00	64·00	0	0
9	25·00	36·00	0	0
10	26·00	28·00	0	0
mean±S.D.	32·0±9·0	45·0±11·7		

Fibrinolytic activity was represented by the lysis area (pig heart units) on the human fibrin plate.
* Refers to the cases with the faint lysed area, though others showed the punched-out lysed areas.

TABLE III.—*Fibrinolytic Activity of CSF and Plasma Before and After Pneumoencephalography (5 Cases)*

Fibrin plate	Human fibrin plate				Bovine fibrin plate			
	Non-heated		Heated		Non-heated		Heated	
	Before	After	Before	After	Before	After	Before	After
CSF	10±11·7	32±5·6	0	0	4±8·3	6±12·4	0	0
CSF eugl.	26±3·4	37±10·2	0	0	6±8·2	10±17·8	0	0
CSF + SK	—	—	—	—	169±28·0	192±30·7	45±20·0	38±22·4
CSF eugl. + SK	—	—	—	—	202±38·2	226±43·4	—	—
plasma	21±27·5	63±25·0	0	0	7·4±6·1	35±30·2	0	0
plasma eugl.	114±19·5	133±27·3	13±14·7	34±45·0	60±28·6	82±22·7	46±21·6	54±22·2
plasma + SK	—	—	—	—	349±59·0	479±78·5	112±65·3	126±26·0
plasma eugl. + SK	—	—	—	—	454±57·8	558±94·7	145±34·5	86±28·4

eugl. refers to euglobulin.
SK refers to streptokinase.
Mean ± SD.

FA of the human brain

The activity of tissue activator differs from organ to organ in the same individual. Uterus, adrenals, lymph nodes, prostate and thyroid were said to have high activities, and little or no activity was found in testes, spleen and liver. Of moderate activity were lungs, ovaries, pituitary, kidneys and skeletal muscles according to Albrechtsen (1957), Ende *et al.* (1961) and Sandberg *et al.* (1964). There seemed to be low activity in brain and high activity in meninges and choroid plexus as our results revealed.

There are considerable variations in the FA of the same organ from different persons. The human brains also revealed moderate individual differences of FA as shown in Fig. 1 and Table I, as Albrechtsen (1957) and Sandberg *et al.* (1964) recognized in other organs.

Alteration of the FA of the human brain with ageing

The difference of the FA of the brain tissue in the newborn infant and adult was revealed by Astrup's method as shown in Fig. 1 and Table I. FA was lower

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in the newborn brain than in the adult one, though Albrechtsen (1957) reported no correlation between activator concentration in brain tissue and age. In the periods of the newborn and infancy, FA seemed to develop with ageing, although the number of the tested cases was small. In the adult brain there were no remarkable alterations with age, except for slight decrease in the older ages. The histochemical method of Todd showed also the same results as mentioned above.

The cause of the increasing FA with ageing was not clear, but the poor enzymatic development in the newborn and arteriosclerosis in the adult might have some correlation with this result. The degree of the vascularization of the brain tissue itself was also considered to be one of the causes of such age difference of the FA.

Distribution of the FA in the human brain

The localization of FA was demonstrated by Todd's histochemical method. The lysed zones were located at the vessels of the brain and meninges, and were related, almost exclusively, to small blood vessels in the brain and meninges.

It was said that FA was correlated to vascularization of tissue, but vascularization alone might be no indication of the presence of plasminogen activator (Kwaan, 1966; Astrup, 1966). Moltke (1958) said that the difference in concentration of plasminogen activator in various parts of the pia mater could be caused by variations in the number of blood vessels. Variation of the FA in the various parts of the same brain might be caused by the distribution of the blood vessels, though such variation was not significant.

Meninges and choroid plexus with vascularized connective tissue had higher activity than brain tissue which showed no apparent topographical differences. Cerebral arteries had moderate activity between those of meninges and brain extracts. And the smaller artery and vein seemed to have higher activity than the larger artery. Choroid plexus rich in capillaries had slightly lower activity and the dura mater had slightly lower activity than the pia mater. The extravasated blood in the subarachnoid and subdural spaces does not clot generally in the cases with subarachnoid haemorrhage, subdural hematoma or cerebral haemorrhage ruptured into the ventricle. High FA of choroid plexus and meninges might play a significant role in the liquefaction of the extravasated blood in those diseases.

FA of CSF

It is known that CSF does not contain fibrinolysin, but contains plasminogen activator and proactivator in the patients without intracranial disorders (Tanaka, 1960; Okada et al., 1966). It was said that plasmin appeared in CSF in the cases of purulent and tuberculous meningitis due to pleocytosis, increased permeability of meninges or damage of the brain tissue (Tanaka, 1960). Porter et al. (1966) indicated that CSF contained an incomplete fibrinolytic activator which was converted to a complete activator by incubation with the euglobulin fraction of plasma or with streptokinase. Hellinger and Vogel (1967) found that fibrinolysis was demonstrated in the normal CSF of the patients with intracranial disorders and in the pathologic CSF with abnormal findings. Tanaka (1960) recognized that plasmin in CSF was found neither in normal condition nor in cases with head trauma, while CSF of all cases with head trauma showed increased plasminogen

activator and proactivator in comparison with that of normal condition. Our observations by means of the human and bovine fibrin plates revealed that CSF contained no plasmin, but contained plasminogen activator and proactivator. FA of CSF increased after PEG and both plasminogen activator and proactivator was higher in CSF after PEG than that before PEG.

This increased activity seemed to be derived from the following mechanism, namely (i) fibrinolytic enzymes may be released from brain substance, meninges or choroid plexus, (ii) permeability of meningeal vessels and choroid plexus may be increased by being excited by air in PEG and the CSF will contain serum factor, (iii) proactivator in CSF may be converted into plasminogen activator. Okada et al. (1966) observed that plasminogen activator increased in CSF and brain tissue of dogs with brain oedema after head trauma, but plasminogen activator activities of CSF and brain tissue did not show any significant relation. Porter et al. (1966) discussed that plasminogen activator was probably responsible for the initiation of fibrinolysis in the meningeal spaces, and fibrinolysin did not appear to cross the blood–brain barrier. As both plasminogen activator and proactivator increased simultaneously in CSF after PEG, the third factor was thought to be less possible. In order to elucidate the mechanism of increased FA of CSF, further studies seemed to be necessary.

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